

IN THE CLAIMS:

Claim 1 (currently amended) A recombinant nucleotide sequence of expression cassette OXY-1 of SEQ ID No. 1.

Claim 2 (currently amended) A modified recombinant staphylokinase SAK-2 gene of SEQ ID No. 2.

Claim 3 (currently amended/withdrawn) A peptide sequence of modified recombinant staphylokinase SAK-2 gene[[,]] of SEQ ID No. 3.

Claim 4 (currently amended) A plasmid pRM1 contained in *E. coli* having International Deposition No. BPL-0019.

Claim 5 (currently amended) A plasmid pOXYSAK-1 contained in *E. coli* having International Deposition No. BPL-0020.

Claim 6 (currently amended) A plasmid pOXYSAK-2 contained in *E. coli* having International Deposition No. BPL-0021.

Claim 7 (original) A recombinant *E. Coli* of International Deposition No. 5146, the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, having a plasmid pRM1 of International Deposition No. BPL-0019.

Claim 8 (original) A recombinant *E. Coli* of International Deposition No. 5147, the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, having a plasmid pOXYSAK-1 of International Deposition No. BPL-0020.

Claim 9 (original) A recombinant *E. Coli* of International Deposition No. 5148, the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, having a plasmid pOXYSAK-2 of International Deposition No. BPL-0021.

Claim 10 (currently amended/withdrawn) A process for over-producing staphylokinase and its analogues by modulating level of oxygen level of its growth medium in a host system, said method comprising the steps of:

- a. ~~preparing a piece of DNA carrying genetic information for the production of staphylokinase,~~
- b. ~~modifying 10 amino terminal residues of SAK encoding DNA, wherein Lys6 and Lys8 residues of SAK are changed to small neutral amino acid residues,~~
- c. ~~constructing DNA expression cassette OXY-1,~~
- d. ~~integrating piece of DNA obtained at step (a) or step (b) with the OXY-1 to obtain pOXYPRO,~~
- a. isolating a staphylokinase gene encoding an SAK protein,
- b. modifying codons in the gene encoding Lys6 and Lys8 residues present at an amino-terminal end of the SAK protein to obtain a

modified DNA,

- c. providing the recombinant nucleotide sequence of expression cassette OXY-1 as claimed in claim 1,
- d. integrating the modified DNA obtained in step (b) with the recombinant nucleotide sequence of expression cassette OXY-1 to obtain a product comprising pOXYPRO,
- e. ~~transferring integrated product of step (d) on a plasmid vector integrating the product obtained in step d with plasmid vector PRM 1 to obtain plasmid construct constructs pOXYSAK-1, and pOXYSAK-2 respectively,~~
- f. introducing the plasmid constructs of step (e) into a host ~~systems~~ system,
- g. culturing the host cell for over-production of SAK or its derivatives under high aeration and changing level of oxygen below 5% of atmospheric oxygen level when cell growth reaches ~~to~~ exponential phase to obtain cell mass,
- h. lysing ~~the~~ of the cell mass cells of step (g) to ~~separating~~ separate cell lysate from ~~the~~ cellular debris, and thereby obtaining the staphylokinase and its analogues.

Claim 11 (currently amended/withdrawn) A process as claimed in claim 10, wherein the Lys6 and Lys8 residues of the SAK protein are changed into small and neutral amino acid residues.

Claim 12 (withdrawn) A process as claimed in claim 10, wherein the plasmid vector is a high or medium copy number plasmid.

Claim 13 (currently amended/withdrawn) A process as claimed in claim 10, wherein the host system is selected from a the group comprising consisting of *E. coli*, *Bacillus*, and Yeast.

Claim 14 (withdrawn) A process as claimed in claim 10, wherein the sequence of OXY-1 is modified depending upon the host system.

Claim 15 (currently amended/withdrawn) A process as claimed in claim 11 10, wherein the amino acids acid residues are selected from a the group consisting of comprising Alanine[[,]] and Glycine.

Claim 16 (currently amended/withdrawn) A process as claimed in claim 10, wherein the culturing is in growth medium is comprising Luria Broth (LB) medium.

Claim 17 (withdrawn) A process as claimed in claim 10, wherein culturing the host cell for over-production of SAK or its derivatives at shake flask culture or at fermentation.

Claim 18 (currently amended/withdrawn) A process as claimed in claim 17, wherein comprising culturing the host cell till O.D. 600 O.D.<sub>600</sub> reaches 0.6 to 0.7.

Claim 19 (currently amended) A process as claimed in claim 17, wherein the fermentation is a two-stage fed-batch fermentation.

Claim 20 (currently amended/withdrawn) A process as claimed in claim 10, wherein comprising obtaining the cell mass by centrifugation or filtration.

Claim 21 (currently amended/withdrawn) A process as claimed in claim 10, wherein the lysing of the cells is by a method selected from a the group consisting of comprising sonication, chemical, and mechanics lysis.

Claim 22 (currently amended/withdrawn) A process as claimed in claim 10, wherein comprising separating the cell lysate from the cellular debris by centrifugation.

Claim 23 (withdrawn) A method of dissolving blood clot in a subject in need thereof, said method comprising step of administering pharmaceutically effective amount of streptokinase analogue SAK-2, optionally along with additive(s).

Claim 24 (withdrawn) A method as claimed in claim 23, wherein the additive is selected from a group comprising nutrients consisting of proteins, carbohydrates, sugar, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste, and/or pharmaceutically acceptable carrier, excipient, diluent, or solvent.

Claim 25 (withdrawn) A method as claimed in claim 23, wherein the SAK-2 and additives are in a ratio ranging between 1:10 to 10:1.